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PRINCIPAL INVESTIGATOR: Samuel Toba,, Ph.D.

CONTRACTING ORGANIZATION: University of California
San Francisco, California 94143-0962

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6. AUTHOR(S)
Samuel Toba, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California
San Francisco, California 94143-0962

E-Mail: toba@francisco.compchem.ucsf.edu

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13. ABSTRACT *(Maximum 200 Words)*

Matrix metalloproteinases (MMPs) is an important new class of therapeutic targets for the treatment of diseases characterized by excessive extracellular matrix degradation and/or remodeling, such as cancer. The inhibition of the MMPs enzymes can serve as disease-modifying agents. MMPs inhibitors help to prevent cancer metastasis and angiogenesis, and hence help stabilize the disease condition. In this research, we will apply structure-based drug design approach to find novel and selective biological probes of MMPs that may be effective cancer therapies. We have tested and validated the zinc metal ion force field for use in molecular docking, and accurately reproduced the experimental binding free energies for complexes of MMPs. Our docking studies of antineoplastic compounds have identified ligand binding preferences for the MMPs and the compounds selected are currently being assayed. In addition, we have investigated the induced fit mechanism in the active site of MMPs. The information obtained will help us in the next stage of designing diverse and focused combinatorial libraries based on a known and a novel scaffold.

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Introduction

Matrix metalloproteinases (MMPs) is an important new class of therapeutic targets for the treatment of diseases characterized by excessive extracellular matrix degradation and/or remodeling, such as cancer. However, to date, most MMP inhibitors that reached clinical trials were withdrawn, in part due to side effects. [1,2] One of the main reasons for these observed side effects is the broad-spectrum nature of the inhibitors. It remains a challenge to identify specific inhibitors for each of the MMPs enzymes.

With the recent proliferation of available crystallographic and NMR structures of many MMPs, it is an advantageous time to apply methodologies of structure-based design to help discover potent and selective inhibitors. In addition, the availability of the numerous good resolution MMPs structures allows us to build accurate homology models of other MMPs enzymes of therapeutic significance. We have previously proposed the use of stromelysin-1 (MMP-3) and MT1-MMP (MMP-14) in our study. However, we have eliminated MMP-14 enzyme from this study due to the unexpected difficulties encountered in obtaining the purified enzymes of MMP-14 as well as in reproducing the experimental assays. These difficulties are due to the membrane bound nature of the MMP-14 enzyme. [3] We have replaced MMP-14 with other MMPs. Specifically, we have focussed on stromelysin-1 (MMP-3), gelatinase-B (MMP-9) and TNF-α converting enzyme (TACE) [4-6] for the several reasons. First, these enzymes are known to have important roles in breast cancer pathways. [7-11] Second, the structures of MMP-3 and TACE are available through the Protein Data Bank (PDB). [11-13] Although the structure for MMP-9 is not available, the high sequence similarity between MMP-9 and MMP-2 (gelatinase A, PDB structures available) [14,15] will allow us to construct a homology model for the enzyme. In addition, in-vitro biochemical assays are available for these enzymes for experimental validation. Finally, these three selected enzymes have different functional proteolytic activities. Their differences in activities allow them to act as representative enzymes in our investigation and help provide crucial insights into the design of potent and selective inhibitors for each of the MMPs.

Body

In this research, we proposed the use of computational techniques in molecular docking and screening that have been developed in our laboratory. With the presence of a catalytic zinc ion in MMPs, the metal ion in may induce a polarization effect around the active site. Hence, our first goal was to develop an accurate zinc ion molecular mechanics force field representation suitable for use in molecular docking. Several force field parameters have been developed for zinc ions. Specifically, we have identified the non-bonded model parameters developed by Clementi et al. [16] and Stote et al. [17] and the distributed charge model parameters developed by Åqvist et al. [18] as being most suitable for use in molecular docking. We have selected these models because of no constraints are imposed, and because these parameters are transferable to the AMBER [19] molecular mechanics force field used in DOCK. [20] We have performed a series of testing and refinements against these zinc models and force fields on known crystal structures of MMPs enzyme-ligand complexes (MMP-1 and MMP-3). [12,13,21] We found that a small modification to the non-bonded model of Stote et al. [17] makes it most suitable for use in docking and can well reproduce the crystallographic zinc coordination state. We have

refined the van der Waals (ε) value for zinc parameters in Stote et al [17] by reducing the van der Waals value and, hence, allowing softer repulsion between the zinc and the ligand ZBG. With the used of this model, we have accurately reproduced the ligand-protein crystal complex zinc binding distance (~ 1.8-2.0Å).

Consistent with the development of the AMBER force field parameter charges used for the proteins, [19] we have performed a single point ab initio calculation followed by a restrained electrostatic potential calculation to obtain the charges for the truncated ligand and active site residues surrounding the zinc ion. This approach allows the distribution of charges from the zinc ion into the neighboring protein residues and the ligand ZBG, thereby simulating the induced polarization effect. Due to the intensive nature of this calculation, we have tested this against the MMP-1 complex only. [21] We compared the docking results obtained using this model against a calculation that employs Gasteiger and Marsili charges. [22] We find that the docked ligand geometries from this model resulted in higher RMS deviation values compared to the crystal geometry. This may be due in part to the limitations in the DOCK energy score evaluation of using only vdW and electrostatic contributions to the AMBER force field. To apply this model in docking, it may be necessary to include the implicit bond, angle, and torsion terms between the zinc and the coordinating ZBG.

In addition to obtaining the correct binding between the ligand ZBG and the proteins through the use of non-bonded model, we have further gained understanding into the docking parameters necessary for MMPs complexes. We found that the use of ZBG 'anchor-based' docking helps to correctly identify the electrostatic complementarity between the zinc and the ZBG functional groups such as the hydroxamate or the carboxylic acid. With the 'peptidyl-like' ligands, it is also necessary to remove the intra-ligand contribution. Minimization of the unbound ligand alone shows the preference for a 'folded' state, whereas the complexed ligand favors the 'open' conformers in the crystal (compensated by the protein-ligand interaction). Hence, we find that the use of only intermolecular energies for the protein-ligand scoring in DOCK allows us to better reproduce the crystal ligand geometry. An unexpected difficulty was encountered in the MMP-3 complex[13] (PDB identifier 1HFS) in our test case. We were not able to dock a known inhibitor into the MMP-3 active site due to the repulsion between the active site Glu-115 residue (numbering by 1HFS) with the carboxylate ZBG of the ligand. Upon closer inspection, we found that the crystal structure of 1HFS was solved through the isomorphous replacement method from another MMP-3 complex[12] (PDB identifier 1SLN). This resulted in unfulfilled interactions between the Glu residue and its ligand in 1HFS structure. Replacing the 1HFS structure with the 1SLN structure in our docking resulted in good ligand binding geometries.

It is well known that the role of water molecules and the desolvation effect during protein-ligand binding plays a critical role in determining the structure and free energy of the complex. In this research, we have modeled the inclusion of solvation in ligand binding through the use of Generalized Born / Surface Area (GB/SA) continuum solvent model.[23] In our tests with MMPs, we have made significant progress in applying the GB/SA scoring in DOCK for solvation correction. We have made three modifications to the GB/SA model: the elimination of the cavity penalty term, the explicit correction for a bound water in the native protein, and the reduced dielectric treatment for the proteins'

active site cavity. Applying these modifications to correctly model the mechanism of protein-ligand binding in MMPs, we were able to closely reproduce the known experimental binding free energies of the MMPs complexes.

Having obtained a good insight into the parameters used and the docking protocols necessary to reproduce crystallographic data, we extended our docking comparison to include a series of known MMP-1 and MMP-3 inhibitors and commonly occurring non-cytotoxic drugs as negative controls. In both cases, we were able to selectively distinguish the known inhibitors from the other drugs based on DOCK free energy score. Our database docking search of ~300 known antineoplastics agents shows the preference for many pyrimidine-like molecules for MMPs. Currently, these compounds are being assayed in the laboratory of Zena Werb (UCSF).

Our docking study of the patented molecules (~150) as inhibitors of TACE have further given us valuable insight into the induced fit mechanism within the MMPs protein upon ligand binding. The understanding obtained from this mechanism will greatly help us tailor our design of inhibitors for selectivity. Through the use of docking, comparative modeling and molecular dynamics (MD), we have identified the regions of high mobility within the TACE active site. Our comparison of the crystallographic thermal factor between the native and the complexed structure of Adamalysin[24-26] (closest protein to TACE; native crystal structure of TACE is not available) have identified the 'bottleneck' region as being the loop and helix around the S1' pocket. Our molecular dynamics simulations of TACE further confirmed this finding and shows ~ 4Å of mobility for the same region in S1'. Other studies of MMPs inhibitors have similarly reported the observed variability in sidechains at the P1' site of the peptidyl-ligand.[27-29] Our test with the use of mutations to Alanine for selected 'blocking residues', coupled with the 'soft-docking' approach have shown good promise as an ad-hoc approach to estimate the induced fit. We are currently performing extended MD simulations on the native and complexed TACE. The protein trajectories obtained from this MD simulations would allow us to explore the different protein structural variability that can be applied to the next stage of this research.

In accord with the tasks outlined in the approved Statement of Work, concurrently we are in the progress of designing several virtual diverse combinatorial libraries based on thiadazole urea scaffold and aminomethyl benzimidazole scaffold. Both of these scaffolds have shown promising inhibitory activity (millimolar to micromolar) for MMPs based on the initial in-vitro assays. The designed libraries will be synthesized and assayed by our collaborator at University of California Berkeley in the laboratory of Dr. Jon Ellman. While the binding mode for thiadazole urea scaffold with the zinc ion has been proposed by Jacobsen et al. [30], the binding mode for the benzimidazole scaffold remains unknown. Through the design, synthesis and assay of the *diverse* combinatorial libraries, we will obtain a structure activity relationship and hence identify the binding mode and/or improve the ligand ZBG. This validation of results between experiment and theory provides a direct feedback mechanism for further ligand design based upon experimental measurements. We will then apply this information to the next stage of our design of *focussed* combinatorial libraries using the most recent library design technologies developed in our laboratory[31] for each MMPs enzyme targets. The lead compounds

obtained from focused libraries for each target can then be further optimized for selectivity.

Conclusion

We have tested and validated the zinc metal ion force field for use in molecular docking. Our docking studies of antineoplastic compounds have identified ligand binding preferences for the MMPs and the compounds selected are currently being assayed. In addition, we have investigated the induced fit mechanism in the active site of MMPs. The information obtained will help us in the next stage of designing diverse and focused combinatorial libraries based on a known and a novel scaffold. Our research is in good progress and is consistent with the proposed goals of finding novel and selective biological probes of MMPs that may be effective cancer therapies.

Key Research Accomplishments

- Tested and validated zinc metal ion force fields against a set of known inhibitors of MMPs using the bonded-model approach. Reproduced the experimental binding free energies of known MMP inhibitors using the modified Generalized Born/Surface Area scoring function.
- Obtained insight into the role of zinc-bound water molecule in the catalytic mechanism of MMP enzymes, and its effect in the continuum solvation model approximation.
- Applied techniques in molecular docking, comparative modeling and molecular dynamics to identified regions of high mobility in the TACE enzyme and the mechanism of induced fit in MMP catalytic site.
- Performed database docking of antineoplastic agents from the Comprehensive Medicinal Chemistry (CMC) database against MMP-1 and MMP-3. Identified pyrimidine-like molecules as potential high-affinity ligand scaffold for MMPs. (experimental assay in progress)
- Set-up collaborative effort with the laboratory of Dr. Jon Ellman (UC Berkeley) for synthesis of combinatorial libraries based on thiadazole urea (known) scaffold and aminomethyl benzimidazole (novel) scaffold.

Reportable outcomes

- Submitted research poster presentation for the Gordon Research Conference in Matrix Metalloproteinases in May 13-18, 2001 (accepted)

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Design and Docking of Combinatorial Libraries Against MMP Targets

S. Toba, I. Nakanishi, I. D. Kuntz
University of California San Francisco
Department of Pharmaceutical Chemistry, Box 0443
San Francisco, CA 94143

Abstract:

The use of combinatorial chemistry has made a much larger number of compounds accessible to the medicinal chemist. To effectively sample this larger chemical space, a means for rapid identification and design of directed libraries is needed. We present a structure-based method for the virtual screening of multiple libraries against a family of MMPs. Coupled with the advent of structural genomics, the design of family-directed libraries has the potential to parallelize and accelerate the lead discovery process.

The method consists of three main stages: docking the scaffold, selecting the best substituents at each site, and comparing the resultant libraries. The core "divide-and-conquer" algorithm for side chain selection provides a way to explore many substituents with a linear time dependence. We have applied our method to the collagenase, gelatinase and TACE proteins. We show that the scaffold docking procedure, in conjunction with a novel vector-based orientation filter, reproduces crystallographic binding modes. We tested the substituent selection procedure, an implementation of the generalized Born / buried surface area (GBSA) method, by comparing to known experimental binding data for inhibitors with different P1' substituents. Finally, we demonstrate the application of the method to analyze the specificity and selectivity of the different scaffold libraries against the different protein targets.